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MAINTENANCE OF PREGNANCY IN THE HYPOPHYSECTOMIZED RABBIT BY THE ADMINISTRATION OF OESTRIN

By J. M. ROBSON

From the Department of Pharmacology, University of Edinburgh

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It is well known that hypophysectomy in the rabbit during the later stages of pregnancy is followed within 24–48 hr. by the expulsion of the uterine contents. That this is due to the cessation of the luteal function which follows withdrawal of the pituitary activity is indicated by the facts that either replacement of the luteal secretion by the injection of progesterone, or maintenance of the luteal function by the administration of gonadotropic hormone, will prevent abortion in such hypophysectomized animals and lead to the continuation of pregnancy [Robson, 1937*a*].

More recent work has shown that the luteal function in the hypophysectomized pseudo-pregnant animal can be maintained by the administration of crystalline oestrone or oestradiol [Robson, 1937*b*], and the question then arises whether such a method of maintaining the luteal function is effective in preventing abortion in animals hypophysectomized during a true pregnancy.

Methods

The mating of rabbits was observed, and on the 21st day of pregnancy the pituitary was removed by the orbital approach [Firor, 1933] and the completeness of the operation was checked by subsequent macroscopic examination of the sella. Any minute pieces of hypophysis which might chance to be left, embedded in blood clot, were presumed to have no recognizable activity. In two animals (821, 826) mating was not observed, but the duration of pregnancy was estimated from the size of the foetuses.

Crystalline oestrone, oestradiol, and oestradiol benzoate were administered in solution in oil. In one experiment (902) the synthetic

oestrogenic substance, triphenyl ethylene, which exerts a prolonged oestrogenic action [Robson & Schönberg, 1937] was administered in solution in oil. Doses are shown in Tables I and II. Injections were given twice daily (morning and evening). The course of gestation was followed by daily weighing and palpation, and laparotomies were occasionally performed. The dissection of the mammary glands for weighing was performed according to the method described by Hammond & Marshall [1930].

Results

In the first group of experiments (Table I) oestrone or oestradiol was used and in ten out of eleven animals the administration of the oestrogen prevented abortion. In no animal, however, was normal pregnancy maintained up to full term and only in three were live foetuses found in the uterus 5–6 days after hypophysectomy. In the other eight animals the foetuses were found dead and in various stages of reabsorption when laparotomy was performed 4–6 days after hypophysectomy. As can be seen from Table I various doses of the hormone were used in an attempt to determine whether an optimum dose for the maintenance of pregnancy could be found, but this was not successful. The higher doses occasionally produced rapid death and reabsorption of the foetuses, an effect presumably due to a toxic action, but when the daily dose was decreased from 5 to 3 μg. (864) this was found not sufficient to prevent abortion which occurred during the course of the injections, 4 days after hypophysectomy. In one control rabbit (942) the ovaries were removed instead of the pituitary on the 21st day of pregnancy and the animal then received twice daily injections of 5 μg. of oestrone. This did not prevent abortion which occurred some 40 hr. after ovarian removal.

These results suggested that toxic effects resulting in the death of the foetuses could not be avoided when sufficient oestradiol was given to maintain pregnancy, and it appeared possible that this was due to the fact that rapid absorption of the oestrogen produced a comparatively high concentration in the blood during short periods immediately following the injections. In order to overcome this difficulty and to produce a more uniform concentration over the 24 hr., oestradiol benzoate was used in a second series of experiments, since it is known that this compound is gradually decomposed and the oestradiol gradually absorbed.

Ten rabbits were hypophysectomized on the 21st day of pregnancy and received injections of oestradiol benzoate twice daily in doses varying from 1 to 10 μg. The results are shown in Table II.

TABLE I. Showing the course of pregnancy in rabbits hypophysectomized during gestation. All animals (except 821, 826) were hypophysectomized 21 days after mating; 821 and 826 were about 20 days' pregnant, as determined from the size of the foetuses

Animal no.	Wt. of animal kg.	Oestrogen injected	Daily dose μg.	Result	Post-mortem findings: Wt. of mammary gland g.	Post-mortem findings: Hypophysectomy
849	3·0	Oestrone	5·0	Two foetuses alive, two partly reabsorbed 6 days after hypophysectomy	29	Complete
876	2·63	Oestradiol	5·0	Two foetuses alive, two partly reabsorbed 5 days after hypophysectomy	28	Complete
844	2·4	Oestrone	5·0	All foetuses alive and *in utero* 6 days after hypophysectomy	31·5	Minute piece left
846	1·9	Oestrone	5·0	All foetuses dead and showing partial reabsorption on 5th day after hypophysectomy	25·5	Small necrotic piece left
837	2·8	Oestrone	5·0	Do.	26·5	Minute piece left
877	2·41	Oestradiol	5·0	Do.	—	Complete
866	2·15	Oestrone	4·0	All foetuses dead and showing partial reabsorption on 6th day after hypophysectomy	25·5	Complete
867	3·17	Oestrone	4·0	Do.	33·5	Complete
864	1·8	Oestrone	3·0	Partial abortion 4 days after hypophysectomy. Remaining foetuses *in utero* and reabsorbing	—	Minute piece left
826	2·4	Oestrone	10·0	All foetuses dead and reabsorbing 4 days after hypophysectomy	—	Minute piece left
821	1·6	Oestrone	10·0	Do.	—	Complete
902	2·06	Triphenyl ethylene	20 mg.	All foetuses dead and showing reabsorption on 5th day after hypophysectomy	—	Complete

Note. Fragmentary remains of the hypophysis are presumed to be without function.

TABLE II. Showing the fate of the pregnancy in rabbits hypophysectomized on the 21st day of pregnancy and then injected with oestradiol benzoate

Animal no.	Wt. kg.	Daily dose of oestradiol benzoate μg.	Period of administration days of pregnancy	Result	Post-mortem findings: Wt. of mammary gland g.	Post-mortem findings: Hypophysectomy
950	3·0	2·0	21–29	Foetuses normal on the 27th day (laparotomy). One live foetus and two dead foetuses born on the 32nd day; four live foetuses *in utero*	41	Complete
952	3·3	2·0	21–28	Foetuses normal on 28th day (laparotomy). One live and five dead foetuses born on 30th day; one dead foetus *in utero*	57	Complete
958	2·9	2·0	21–28	Three dead and early reabsorbed foetuses aborted on 30th day. One? live foetus on night 30–31st day; two live foetuses *in utero*	44	Minute necrotic piece left
959	3·4	2·0	21–28	One dead + three live + four? live foetuses born on 30th day	52	Complete
980	1·6	1·5	21–25	Abortion on 4th day after hypophysectomy	—	Complete
982	3·1	1·5	21–28	Live foetuses born on 30–31st day	38	Complete
983	1·8	1·5	21–28	Foetuses born on 30th day. Parturition rather difficult	18	Complete
967	2·8	1·0	21–28	Dead on morning of 32nd day. Uterus contained nine foetuses which were probably alive at time of death of mother	43	Complete
971	2·55	1·0	21–26	Two foetuses aborted on 26th day and one on 29th day	—	Complete
978	2·4	10·0	21–25	Foetuses dead and showing signs of reabsorption on 4th day after hypophysectomy	—	Complete

Note. The fragment of hypophysis (958) is presumed to be without function.

Four animals received 2 μg. of oestradiol benzoate per day from the 21st to the 28th (952, 958, 959) or to the 29th day of pregnancy (950). The injections were discontinued after the 28th or the 29th day, as it was expected that cessation of oestrin administration would be followed by a cessation of the luteal activity and by parturition, and this proved to be so. In all these animals parturition occurred within 2–3 days of the last injection. Parturition appeared to be normal and some live foetuses were born in all these animals, though some others were born dead. These results indicate that the administration of oestradiol benzoate can maintain pregnancy up to full term and that cessation of its administration is within a short time followed by a normal parturition.

Since these experiments were not wholly successful, in that a number of the foetuses had died by the time of parturition, attempts were made to improve the results by using different doses of the hormone. In one experiment (978) the dose was increased to 10 μg. per day, but, though abortion did not occur, the foetuses showed acute signs of absorption within 4 days of hypophysectomy (i.e. on the 25th day of pregnancy), indicating that toxic actions were rapidly being produced and that the dose of the hormone was probably too high.

In two animals the dose was reduced to 1 μg. per day. In one of these (971) abortion occurred during the injection period 5 days after the removal of the pituitary, while in the other (967) the animal died on the 32nd day of pregnancy, i.e. 4 days after the last injection without having gone into parturition. Examination of the uterine contents strongly suggested that the foetuses were alive and normal at the time when the animal died.

Finally, three animals received 1·5 μg./day (0·5 μg. in the morning and 1 μg. in the evening), and in one of these (982) all the foetuses were born alive 2–3 days after the last injection. In one animal (980) abortion occurred 4 days after hypophysectomy, whilst in the third one (983) parturition was definitely seen to be difficult, and the foetuses were born dead 2 days after the last injection. None of the hypophysectomized animals, in which pregnancy was maintained with oestrin, made a nest.

In order to determine whether a synthetic oestrogen which has previously been shown to maintain the luteal function in the hypophysectomized pseudo-pregnant rabbit [Robson, 1938] would also maintain pregnancy, one animal (902) was hypophysectomized on the 21st day of gestation and, twice daily, received injections of 10 mg. of triphenyl ethylene in oil. Abortion was prevented, but the foetuses were dead

and showed early signs of reabsorption on the 5th day after the pituitary removal.

The corpora lutea were examined microscopically in all cases at the end of the experiment. Their appearance was similar to that seen at the same stage of a normal pregnancy with the possible exception of animal 971, in which there was some suggestion of degenerative change in the corpora lutea. Fig. 1 shows the appearance of the corpora lutea in rabbit 982 which gave birth to a normal litter.

The weights of the mammary glands are given in Tables I and II. In animals going on to full term the weight of the mammary gland is appreciably less as compared with the weight of the mammary gland in normal pregnancy. No milk could be expressed from the glands of the rabbits going on to full term though occasionally a small amount of clear fluid was present. A section of the gland of animal 959 is shown in Fig. 2. In some of the animals in which the foetuses were reabsorbing (e.g. 837, 846) a few drops of milk could be expressed from the gland.

Discussion

The results show clearly that when rabbits are hypophysectomized during the later stages of pregnancy, abortion can be prevented by the administration of a suitable quantity of an oestrogen. When the oestrogen used was oestrone or oestradiol the period during which the foetuses remained alive *in utero* was comparatively short and in none of these experiments were the embryos maintained alive up to full term.

The results were, however, much more satisfactory when oestradiol benzoate was used and in some experiments pregnancy was maintained up to full term. Indeed some live foetuses were present at full term in all four animals which received 2·0 μg./day and a normal parturition occurred. An increase of the dose to 10 μg./day resulted in toxic effects which led to a rapid reabsorption of the embryos (animal 980), but when the dose was decreased below 2 μg./day premature expulsion of the uterine contents occurred during the period of injections in two out of five of the experiments, though a normal litter was born at full term in one of the animals which received 1·5 μg./day (982).

These results suggest that oestradiol will maintain a normal pregnancy only when its concentration does not fluctuate outside certain narrow limits. Whilst an increase above this limit results in toxic effects and in death of the uterine contents, a fall below the optimum level may be followed by a premature expulsion of the uterine contents. This may

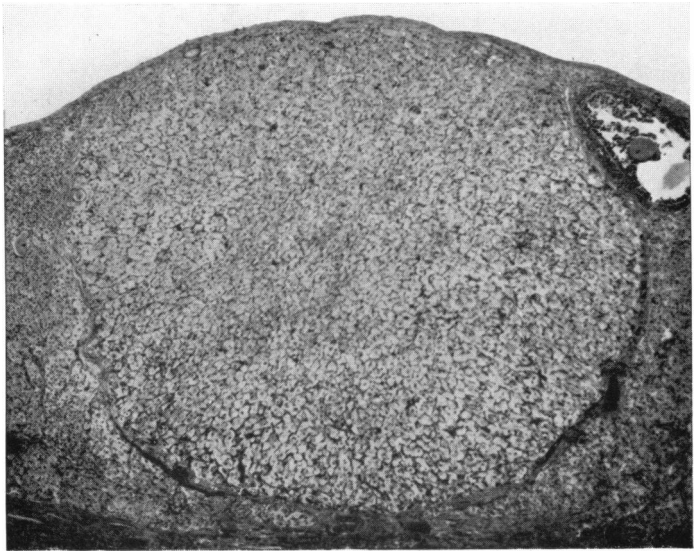

Fig. 1. Showing the condition of the corpus luteum in a hypophysectomized rabbit (982) in which pregnancy was maintained by the administration of oestradiol benzoate. ×38.

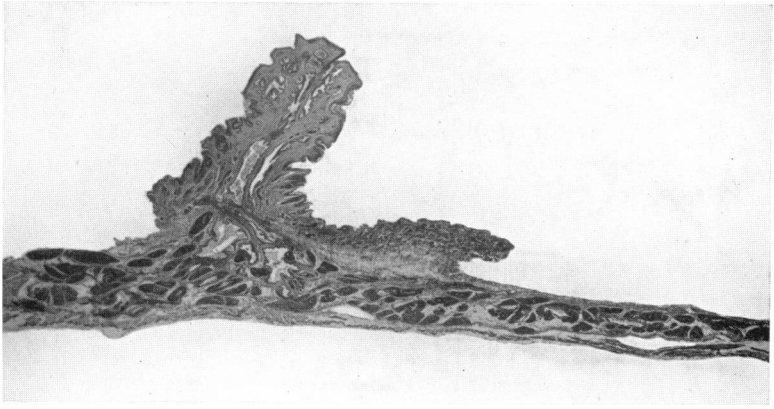

Fig. 2. Section of the mammary gland from a hypophysectomized rabbit (959) in which pregnancy was maintained by the administration of oestradiol benzoate. ×6.

explain the fact that the present experiments were not entirely satisfactory in that (with the exception of one animal (982)) some of the foetuses died *in utero,* even at what appears to be the optimum dose of oestradiol benzoate. It seems reasonable to assume that a more accurate control of the concentration of oestradiol in the blood at the value necessary for the maintenance of pregnancy can much more easily be produced by the secretory activity of the ovary than by the subcutaneous administration, even twice daily, of oestradiol benzoate.

The question now arises as to the part oestradiol plays in the maintenance of a normal pregnancy. That in the rabbit the luteal function is necessary for the maintenance of pregnancy almost up to full term is supported by the findings that cessation of the luteal secretion, either by removal of the ovaries, or by the removal of the pituitary (which is followed by a degeneration of the corpora lutea) produces abortion, and that under both these conditions the administration of the luteal hormone (progesterone) will maintain gestation [Firor, 1933; Robson, 1937*a*; Courrier & Kehl, 1938].

Now it is generally assumed that the factor responsible for the maintenance of the luteal function during pregnancy is a gonadotropic (? luteinizing) hormone acting directly on the corpus luteum. The present experiments raise the question whether oestradiol may not be the factor which maintains the luteal function during pregnancy and this possibility is supported by the previous evidence [Robson, 1938] suggesting that gonadotropic hormone has no direct action in maintaining the activity of the corpora lutea of pseudo-pregnancy, but does so by inducing production of oestrin.

There is evidence in the literature that the placenta exerts an action in the maintenance of the luteal function (see Newton [1938] for a discussion of this question), and Astwood & Greep [1938] have actually extracted a factor from rat placentae which produces an action on the corpora lutea. In view of the evidence presented, the possibility must be considered that the placenta may play a part in controlling the luteal activity through the secretion of oestrin. There is indeed good evidence that the placenta may secrete oestrin in certain species [Newton, 1938].

If it be assumed that the luteal function is maintained during pregnancy by the action of oestrin on the corpus luteum, and that a decrease of oestrin production is followed by a cessation of the luteal secretion, which leads to a termination of pregnancy, then it is necessary to consider how the increase in uterine contractions resulting in parturition is brought about. There is much evidence in the literature that

oestrin plays an important part in activating the uterine contractions at parturition [Robson, 1934; Reynolds, 1937]. Hence it seems difficult to explain how a decrease in oestrin production can occur, allowing the corpus luteum to degenerate, and yet how oestrin can at the same time play an important part in producing the increase of uterine contractions which result in parturition. It must, however, be emphasized that this sequence of events has actually happened in the experiments with oestradiol benzoate described in this paper, viz. the luteal function and pregnancy have been maintained by the administration of oestradiol benzoate, and a cessation of the injections has been followed after 2–3 days by a normal parturition.

A possible explanation is that the concentration of oestrin necessary for the final activation of the uterine muscle is appreciably smaller than that required for the maintenance of the luteal function. Hence, although the decrease in the concentration of oestrin, which occurs in these experiments towards the end of gestation, is sufficient to cause the arrest of the luteal function yet the concentration of oestrin which persists is still adequate to activate the uterine muscle when the antagonistic influence of progesterone is removed by the regression of the corpus luteum.

Summary

Rabbits were hypophysectomized on the 21st day of pregnancy and then injected twice daily with an oestrogen (oestrone, oestradiol, oestradiol benzoate, and triphenyl ethylene). Abortion was prevented in animals receiving an adequate dose.

In none of the hypophysectomized pregnant animals treated with oestrone or oestradiol was pregnancy carried on to full term, though live foetuses were present in some of the experiments 6 days after removal of the pituitary.

More satisfactory results were obtained in the animals treated with oestradiol benzoate: in some of these rabbits pregnancy was carried on to full term and cessation of the injections was followed in 2–3 days by a normal parturition.

It is suggested that the luteal function in the pregnant rabbit may be controlled by oestrin and that a decrease in the production of oestrin may be followed by a regression of the luteal activity and by parturition.

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